Serotonin manipulations and social behavior

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CHAPTER 7

GENERAL DISCUSSION

7.1 Summary of the main findings

This thesis focused on the role of serotonin in regulating social behavior in individuals at risk for major depressive disorder (MDD). In the commentary paper (Hogenelst et al., 2015b) (Chapter 3), I evaluated studies involving manipulation of the human brain serotonin system and including an assessment of social behavior. To date, these studies have mostly been laboratory-based and included computer tasks, observations by others, or single-administration self-report measures. Most laboratory studies performed so far, though designed to inform about the role of serotonin in aspects of social interaction, used measures with low ecological validity. The relevance of the obtained data for real-life interaction often remains unclear. For example, many studies involved computer tasks that assessed the recognition of static facial emotion expressions, rather than actual social behavior. Few studies have used semi-naturalistic assessments of social behavior in the lab and fewer still have assessed social behavior in everyday life. The commentary provided suggestions for several laboratory measures for assessing social behavior with higher ecological validity, and ecological momentary assessment for measuring everyday social interactions. Examples of such methods were applied in Studies 1 and 2, respectively (Chapters 4-6).

In Study 1, men and women with (FH+) or without (FH-) a family history of MDD underwent an acute tryptophan depletion (ATD) procedure, to rapidly and temporarily lower their brain serotonin levels. I primarily assessed the effects of ATD on empathic accuracy (EA), which refers to individuals' ability to correctly identify the internal states of others and is critical to social interactions (Zaki et al., 2009). EA was assessed using videos of targets discussing autobiographical emotional events. Multimodal and semi-naturalistic stimuli (i.e. videos) increased the ecological validity of our EA task compared to most previously used facial emotion recognition tasks (Zaki & Ochsner, 2009). While ATD has previously been found to alter facial emotion recognition (e.g., aan het Rot et al., 2010; Harmer et al., 2003), I found that ATD did not significantly affect EA. However, ATD significantly lowered oxytocin levels. In Chapter 4 (Hogenelst et al., 2015c), I suggested that while isolated aspects of social functioning (e.g., facial emotion recognition) may be impaired following ATD, performance on tasks that more closely resemble real-life social functioning seems to remain intact. I also proposed that lowering oxytocin levels may not impair EA even though increasing oxytocin by intranasal administration improves EA in some individuals (Bartz et al., 2010).

In Study 1, we secondarily assessed the effects of ATD on speech and behavioral mimicry. Speech characteristics are important components of social skills (Segrin, 2011) and mimicry is pervasive in virtually all social interactions, stimulating rapport, liking, and prosocial behavior and serving as social glue (Chartrand & van Baaren, 2009).

Speech and mimicry were also assessed semi-naturalistically, both in response to seeing a woman during a video and during conversations with me, the experimenter. ATD reduced the time participants waited before speaking, but otherwise did not significantly affect speech and behavioral mimicry. In Chapter 5 (Hogenelst et al., 2015d), I speculated that the shortened time before speech during ATD may have indicated a more impulsive speech pattern. Otherwise, the results suggested that an acute reduction in brain serotonin may in never-depressed individuals have little impact on verbal and non-verbal behavior during social interactions.

Study 2 focused exclusively on individuals with a family history of MDD. In this study I assessed the effects of repeated administration of the serotonin precursor tryptophan on social behavior and mood in everyday social interactions. Ecological momentary assessment (EMA), which allows sampling from multiple daily life settings (Mehl & Conner, 2012b), was used to repeatedly assess participants' social behavior and mood during their naturally occurring social interactions. A novel aspect of the study was the assessment of social cognitions at the end of each test day. When participants were taking tryptophan, they unexpectedly reported higher levels of quarrelsomeness and lower levels of agreeableness, specifically when they were at home. Tryptophan improved mood regardless of location, and in different social interactions than in the interactions in which the behavioral effects occurred. Further, negative social cognitions were lower during tryptophan when tryptophan was given second and lower during placebo when placebo was given second, which may indicate that negative social cognitions decreased over time. In Chapter 6 (Hogenelst et al., 2015a), I suggested that the tryptophan-induced increase in quarrelsomeness and decrease in agreeableness at home might have been a way for FH+ individuals to enhance control in their social interactions. Experiencing more control might then lead to mood improvement spilling over in other interactions.

7.2 Meaning of the main findings

As I mentioned in the Introduction and Rationale of this Doctoral Thesis (Chapters 1 and 2), Studies 1 and 2 were conducted to gain more insight in the role of serotonin in the regulation of social interaction in individuals at familial risk for MDD. More specifically, studying the effects of acute reductions in brain serotonin on social functioning in FH+ individuals may help explain how low levels of serotonin may contribute to the occurrence of MDD. Studying the effects of repeated increases in brain serotonin on social functioning in FH+ individuals may help explain the effects of SSRI treatment in MDD patients (see also Figure 3, page 20 in Chapter 1). Indeed, changes in social functioning can lead to changes in mood. Reduced social functioning (e.g., lower empathy, increased

use of negative words) may hamper the acquisition of positive social reinforcement and result in more social isolation, which in turn may negatively influence mood (Hames et al., 2013). In contrast, increases in positive social behavior will invite more positive social responses from others, initiating a cycle or iterative process resulting in improved mood. Though the change in mood after each social interaction will be small, after many interactions over longer periods of time, especially when with the same person, the effect should be much greater and become clinically relevant (cf. Young et al., 2014).

Previous studies indicate that FH+ individuals may exhibit an innate vulnerability of their serotonin system which may negatively influence the regulation of their affective states, the processing of emotional information, and responses to social stimuli (i.e., facial emotion expressions) (Feder et al., 2011; van der Veen et al., 2007). Nevertheless, the results of Study 1, described in Chapters 4 and 5, show that when social information is presented more realistically, acutely low levels of serotonin may not affect social behavior in FH+ individuals. However, as I will also discuss in paragraph 7.3, there are other explanations for the lack of significant effects. In paragraph 7.4 I will then elaborate on the behavioral effects of tryptophan administration in FH+ individuals.

As mentioned in the Introduction and in Chapter 6, Young and colleagues (2014) recently proposed that serotonergic antidepressants might work by acutely increasing agreeableness and reducing quarrelsomeness in depressed individuals and that this may result in a gradual mood improvement. However, the results of Study 2 imply that serotonergic antidepressants might improve mood, but at the same time increase quarrelsomeness and decrease agreeableness in some MDD patients when they start treatment. Based on the principle of complementarity in social interactions (Carson, 1969; Kiesler, 1983; Moskowitz et al., 2007; Sadler et al., 2009), an increase in quarrelsome behavior may elicit more quarrelsome behavior in others. While patients' mood may improve, the effects on social interaction would potentially be less desirable, and might negatively affect patients' interpersonal relationships as treatment progresses.

The idea that serotonergic antidepressants might increase quarrelsomeness and decrease agreeableness in MDD patients is however in contrast with previous studies assessing the effects of serotonergic antidepressants on social functioning in MDD patients. For example, in one placebo-controlled study, 8 weeks of SSRI treatment increased extraversion, a personality trait characterized by high levels of affiliative behavior, presumably also including agreeableness (Tang et al., 2009). In another study, 8 weeks of SSRI treatment reduced subjective hostility, and therefore perhaps also quarrelsomeness (Farabaugh et al., 2010). Yet the extent to which changes in extraversion and hostility, assessed once before and once after treatment, reflect actual behavioral changes in their daily lives is unclear. Future studies are necessary to assess the effects of increas-

es in serotonin, induced by serotonergic antidepressants or tryptophan administration, on everyday social behavior in MDD patients. Suggestions for future studies are made in Chapter 8.

Implications for clinicians

In the Introduction (Chapter 1) I stated that in the last few decades, psychiatry has put an emphasis on the neurobiology of mental disorders (see also Haack & Kumbier, 2012). This has generated critique from researchers in the field of clinical psychology. For example, Bentall (2009) argued that psychological distress in human beings is usually caused by unsatisfactory relationships with other human beings and that warmth and collaborative relationships, rather than medication, are necessary to promote psychological healing. More specifically, it has been suggested that warm and collaborative relationships between patients and mental health care practitioners contribute to a good therapeutic alliance which is key to treatment success (also see Horvath, Del Re, Fluckiger, & Symonds, 2011).

While it may be the case that psychological distress may be better treated by psychological healing rather than medication, the results of Study 2 (Hogenelst et al., 2015a) and of previous studies (aan het Rot et al., 2006; Farabaugh et al., 2010; Moskowitz et al., 2001; Tang et al., 2009) suggest that MDD patients who start with antidepressant treatment may show changes in their social behavior. To date, the idea that medication may have direct effects on social behavior of patients has received scant attention in both psychiatry and clinical psychology. Yet in clinical practice it may be relevant to understand how certain medications can affect the interpersonal relations of patients and in what contexts behavioral changes are likely to emerge. Clinicians may be better able to inform persons in the patients' social environment (e.g. family and friends) regarding the changes that may occur in the patients' social behavior as a consequence of medication. Likewise, medication-induced changes in social behavior may affect the dynamics of the therapeutic relation between clinician and patient. If, on the one hand, antidepressants decrease quarrelsomeness and increase agreeableness in some MDD patients (cf. Young et al., 2014), this may then result in more positive responses from the clinician and positively contribute to treatment outcome. If, on the other hand, antidepressants indeed increase quarrelsomeness and decrease agreeableness in some MDD patients when they start treatment (cf. Study 2), this may then elicit counterproductive behavior from the clinician and negatively influence treatment outcome.

Overall, the results of Study 2 (Hogenelst et al., 2015a) contribute to the idea that researchers and clinicians should consider the interpersonal effects of medication in the treatment of psychiatric disorders.

7.3 Discussion of the methods of Studies 1 and 2

Participants

For both studies described in this Doctoral Thesis, we recruited never-depressed FH+ individuals. FH+ was defined as having a first-degree relative diagnosed with MDD. The reason was that FH+ individuals are thought to be at increased risk of developing MDD compared to FH- individuals (Sullivan et al., 2000). As I mentioned in the Introduction (Chapter 1) and in the papers describing Study 1 (Chapters 4 and 5), a reduction in brain serotonin levels may have a more negative influence on the regulation of the affective state and possibly social behavior in FH+ individuals than in FH- individuals. (Neumeister et al., 2002; van der Veen et al., 2007; Firk & Markus, 2008; Feder et al., 2011). Conversely, increasing brain serotonin levels using tryptophan administration may influence social behavior and mood of FH+ individuals more than FH- individuals. This idea was based on the findings that tryptophan administration has more pronounced effects on social behavior in high trait irritable individuals (aan het Rot et al., 2006), who are, like FH+ individuals, known to be at increased risk for MDD (Conner et al., 2003), than in a random sample of healthy volunteers (Moskowitz et al., 2001). Studies involving FH+ individuals may further our understanding of the role of serotonin in regulating mood and social behavior in MDD patients. Therefore, a strength of Studies 1 and 2 is that we included FH+ individuals.

However, there are a number of other factors that also influence depression risk. Amongst others, these include gender, genetic factors, aspects of personality such as neuroticism and hostility, and having encountered early life stress. The prevalence of MDD is twice as high in women as in men (Gutierrez-Lobos, Scherer, Anderer, & Katschnig, 2002; M. M. Weissman & Olfson, 1995). Further, women have been shown to be more susceptible to mood lowering following ATD than men (Booij et al., 2003). Further, there is evidence that the effects of ATD on facial emotion recognition may be different for men and women. In a study including 18 healthy female and 20 healthy male volunteers, ATD impaired the recognition of fearful facial expressions in women, but not in men (Harmer et al., 2003). In studies described in Chapters 4-6, gender was therefore controlled for in the analysis. Post-hoc, we also examined whether gender moderated the effects of the serotonin manipulation on social behavior and mood. No such moderating

effects were found. Thus, gender did not significantly influence the effect of serotonin manipulation on social behavior and mood.

The effects of the serotonin manipulations may also have been moderated by polymorphic genes involved in serotonin neurotransmission. For example, previous studies suggest that individuals possessing one or two copies of a short (s) allele relative to those homozygous for a long (l) allele of the promotor-linked region of the gene coding for the of the serotonin transporter (5-HTTLPR) may be more susceptible to the negative effects of ATD on mood (Neumeister et al. 2002, 2006; Roiser et al. 2007; Walderhaug et al. 2007) and facial expression recognition (Marsh et al., 2006). Although not presented in Chapters 4-6, we also assessed this polymorphism in the 5-HTTLPR genotype in Studies 1 and 2. In Study 2, 5-HTTLPR genotype did not moderate the effects of tryptophan on social behavior and mood. In Study 1, 5-HTTLPR genotype did not moderate the effects of ATD on social behavior, but it did moderate the effect of ATD on mood. Specifically, positive affect was significantly lower after ATD than after placebo in s-carriers, whereas no significant effect of ATD was observed in ll-homozygotes. Analyses within each FH group subsequently revealed that only FH+ s-carriers reported less positive affect following ATD. There was no significant effect of ATD on positive affect in FH- individuals or in FH+ ll-homozygotes. These results replicate previous results by Neumeister et al. (2002) and provide additional evidence for the idea that possessing one or two copies of the short variant of a 5-HTTLPR and a positive family history of depression may be additive risk factors for mood change following ATD. It remains to be seen whether a 5-HTTLPR genotype moderates the effects of ATD on social functioning other than facial emotion recognition.

We did not assess personality traits such as neuroticism. Neuroticism is a known risk factor for depression (Lahey, 2009; Vinkers et al., 2014). Individuals high in neuroticism often exhibit heightened emotional reactivity to stressful events, tend to be more self-critical and overly sensitive to the criticism of others, and generally report to experience low social support (Lahey, 2009). They have also been shown to report relatively high levels of quarrelsome behavior and low levels of agreeable behavior and exhibit high levels of unpleasant affect during everyday social interactions in an EMA study (Cote & Moskowitz, 1998). These studies suggest that variations in neuroticism may be associated with variations in behavior and mood in an interpersonal context. The extent to which neuroticism moderates the effect of serotonin manipulation on social behavior has not been studied directly. However, in individuals with increased levels of irritability, which is a facet of neuroticism (Costa & McCrae, 1992), tryptophan administration decreased quarrelsomeness, increased agreeableness, and improved mood (aan het Rot et al., 2006). Further, individuals with increased levels of irritability are also more prone to

show increased aggressive feelings and behaviors in response to ATD (Bjork et al., 2000, Cleare and Bond, 1995; Doucherty 1999; Marsh 2002). Together, previous research suggests that neuroticism may moderate the effect of manipulations of serotonin on social behavior. Yet, previous studies showed that the levels of neuroticism were not associated with the mood change after ATD in healthy individuals (Stewart, Deary, & Ebmeier, 2002) and remitted MDD patients (Booij & Van der Does, 2007) implying that in Study 1 neuroticism may not have moderated the effect of ATD on mood.

Lastly, we did not assess information on participants' early life stress (ELS) experiences. Studies have demonstrated ELS to be related to the onset and severity of depression (Kendler, Kessler, Neale, Heath, & Eaves, 1993; Kessler, 1997). ELS may predispose individuals to MDD via epigenetic changes in serotonergic and hypothalamic–pituitary–adrenal axis activity (Booij, Wang, Lévesque, Tremblay, & Szyf, 2013) and thereby negatively affect emotional processing (Heim, Plotsky, & Nemeroff, 2004; Gatt, Nemeroff et al., 2010; Gatt, Williams et al., 2010; Pechtel & Pizzagalli, 2011) and possibly also the processing of socially relevant information (Grant, Cannistraci, Hollon, Gore, & Shelton, 2011). If ELS contributes to depression risk through epigenetic changes in the neurobiology underlying social functioning, then the negative effects of ELS on social functioning may emerge only after challenge of the underlying neurobiological circuitry (e.g., after ATD). If we had included a group of FH+ individuals with varying levels of ELS we would have been able to test this idea in Study 1.

Manipulation of serotonin

ATD can be used to selectively study the effects of acute reductions in brain serotonin (Young, 2013a). ATD is an experimental procedure that has gained insight into the effect of low serotonin in the regulation of a range of emotional, cognitive, and behavioral functions (Booij et al., 2003; Mendelsohn et al., 2009; Young, 2013c).The ethical aspects of ATD have recently been discussed (Young, 2013a). Further, few ATD studies carried out to date have reported adverse events, which probably owes to the short duration of the metabolic change. Furthermore, as was done in Study 1, giving 1 gram of tryptophan at the end of a study day rapidly restores serotonin levels (Young, 2013a).

It is generally assumed that ATD decreases serotonin function, meaning a decrease in serotonin synthesis that subsequently decreases the release of serotonin onto postsynaptic receptors (Young, 2014). However, it should be noted that while ATD has been shown to reduce basal serotonin synthesis in the human brain (Nishizawa et al., 1997), and stimulated serotonin release in the rat brain (Stancampiano et al., 1997), there is no direct evidence that ATD decreases serotonin release in humans (Young, 2013a).

The negative findings of Study 1 (Chapters 4 and 5) should be interpreted in light of this notion.

Moreover, even if ATD reduces serotonin release in humans, there may be compensatory mechanisms that prevent ATD from affecting social behavior. There is evidence to suggest that this may occur. One past study in individuals recently remitted from MDD examined whether the ATD-induced depressive mood was mediated by the density of the serotonin-type 2 receptor in the brain assessed using positron emission tomography (Yatham et al., 2012). In contrast to individuals whose mood worsened during ATD, individuals who did not experience a mood worsening showed a robust down-regulation of serotonin-type 2 receptors over the course of the test session. The authors hypothesized that serotonin-type 2 receptor down-regulation may be a compensatory mechanism to prevent ATD–induced depressive response. In study 1, a similar compensatory mechanism may have prevented an effect of ATD on social behavior.

Whereas short-term reductions in serotonin may not significantly affect social functioning in FH+ individuals (Chapters 4 and 5), long-term reductions in serotonin may have an effect. However, as discussed in the Introduction (Chapter 1), multiple sessions of ATD are considered unethical (Young, 2013b) and there is no alternative. Nevertheless, longer-term increases in serotonin may be investigated by repeated tryptophan administration (Study 2) or by administering e.g. SSRIs.

As mentioned in the Introduction (Chapter 1), using tryptophan to increase brain levels of serotonin has several benefits over serotonergic medication such as SSRIs. Whereas placebo-controlled studies involving SSRIs may suffer from side-effects (e.g., Paul et al., 2007)which increases the risk of unblinding(Crockett & Fehr, 2014; e.g., Paul et al., 2007), tryptophan produces very few side effects (Moskowitz et al., 2001; Thomson et al., 1982), which increases the likelihood of maintaining blind conditions in placebo-controlled studies. In Study 2 (Chapter 6) blind conditions were indeed maintained, as the percentage of participants who were correct in guessing when they were taking tryptophan was not significantly different from chance (Hogenelst et al., 2015a).

At the dose used in study 2, the oral administration of tryptophan has also not been shown to increase serotonin release in humans. Nonetheless, previous studies have found that the oral administration of tryptophan increases serotonin synthesis in the monkey brain (Leathwood & Fernstrom, 1990) and increases levels of 5-hydroxyindoleacetic acid, serotonin's main metabolite, in humans (Young & Gauthier, 1981). In addition, in rats, ingestion of a protein with a high concentration of tryptophan increases serotonin release (Orosco et al., 2004). These studies suggest that tryptophan may also enhance serotonin release in humans.

Assessment of social behavior

A strength of Study 1, is that the EA task involves videos of people (targets) talking about emotional life events (Zaki et al., 2008; aan het Rot & Hogenelst, 2014), whereas previous ATD studies often involved static facial emotion expressions (e.g., aan het Rot et al., 2010; Harmer et al., 2003). Moreover, the task considers the emotional expressivity of the targets. Using video stimuli and considering target expressivity, both increase the ecological validity of the data obtained (Ambadar et al., 2005; Zaki & Ochsner, 2009). However, a disadvantage of the EA task is that the predictive value for everyday social functioning has not been determined. In contrast, for the predictive value of the FER task this has been assessed; a previous study found FER to be unrelated to social functioning assessed with EMA (Janssens et al., 2012). In section 7.4 I will further discuss the EA task in relation to FER tasks.

In Study 1, speech was recorded semi-naturalistically, i.e. towards a camera after watching an unknown woman in a video and towards a male experimenter in a semi-structured social interaction. Mimicry was recorded towards an unknown woman in a video and towards a male experimenter in a semi-structured social interaction. Mimicry was assessed by two independent observers. As mentioned in Chapter 3 (Hogenelst et al., 2015b), the advantage of observational assessment is that observers can provide an accurate assessment of the behavior displayed. Yet, while the assessment of mimicry was fairly naturalistic, the extent to which mimicry towards an unknown woman in a video and towards a male experimenter in a laboratory setting is representative for mimicry in daily life can be debated. Ecological validity would have been better if mimicry had been assessed during social interactions with a known close interaction partner (e.g., friends, family) (see also Chartrand & van Baaren, 2009).

The EMA method used in Study 2 involved multiple assessments per day over two periods of 14 days. As outlined in Chapter 3 (Hogenelst et al., 2015b), repeated sampling allows for aggregation across multiple data points to decrease the error variance in the measure (Epstein, 1979; Moskowitz & Schwarz, 1982; Brown & Moskowitz, 1998) and may therefore be more sensitive to manipulation of brain serotonin levels than the measures used in Study 1. In addition, the EMA method employed in Study 2 takes naturalistic contexts into account. This was of clear value in Study 2, as tryptophan was found to influence behavior specifically during social interactions at home (Hogenelst et al., 2015a).

As I mentioned in Chapter 3 (Hogenelst et al., 2015b), EMA also has its disadvantages. EMA procedures may be more demanding for participants and researchers than the measures used in Study 1. Further, the EMA method used in Study 2 was based on self-report (Hogenelst et al., 2015a). The study therefore depended on participants' moti-

vation and ability to accurately report on their behaviors and feelings (Hogenelst et al., 2015b). In a previous study in which participants were asked to record their own behavior, there was a high correlation with how their behavior was rated by independent observers (Moskowitz, 1990). Moreover, Green et al. (2006) have previously shown that compliance can be high when participants report on their social interactions. This indicates that accurate self-report of behavior is possible. Nevertheless, more objective EMA-type methods are available to assess aspects of social functioning and could have been used in Study 2. For example, everyday EA can be assessed using an EMA method in which both partners in a couple rate their own and the other's feelings in daily life (Gadassi et al., 2011). However, while this method may be more objective than the EMA method used in Study 2, it does not assess actual social behavior in its current form. Another EMA-type method is the electronically activated recorder employed by Baddeley et al. (2013) which can be used to assess speech content. While this method could have been used alongside the EMA method that was used in Study 2, adding an additional EMA procedure might have overburdened participants.

Lastly, the EMA method used in Study 2 involved paper-based social interaction forms. Some researchers have suggested that for EMA studies, using electronic assessment may yield higher compliance compared to using paper-based forms (Stone, Shiffman, Schwartz, Broderick, & Hufford, 2003). However, while compliance can be tested for signal- or time-based designs, for event contingent recording this is more difficult. Nevertheless, backfilling and forward filling of social interaction record forms may be prevented to some degree using clear instructions as was done in Study 2 (Chapter 6).

Data analysis

The covariate analysis in Study 2 indicated that the tryptophan-induced increase in quarrelsomeness and decrease in agreeableness occurred in different social interactions than the interactions in which tryptophan improved mood (Chapter 6). The statistical analysis that was used did not allow disentangling temporal patterns or relationships among social behavior and mood. In other words, it is unclear to what extent tryptophan-induced changes in behavior preceded tryptophan-induced changes in mood or vice versa. Of course it is possible that in Study 2, tryptophan increased quarrelsomeness and decreased agreeableness completely independent of the enhancement of mood. However, previous studies have shown that social experiences and mood can be related over time (Gunthert et al., 2007; Vella, Kamarck, Flory, & Manuck, 2012). In one study, stress resulting from social encounters was not found to be associated with depressive symptoms when assessed at the same time, but did predict depressive symptoms assessed on the next day

(Gunthert et al., 2007). In another study, stress resulting from social interactions predicted subsequent hostile mood, whereas hostile mood did not predict social strain (Vella et al., 2012).

In Study 2 (Hogenelst et al., 2015a), a tryptophan-induced increase in quarrelsomeness and decrease in agreeableness at home may have increased FH+ individuals' sense of control over social interactions at home. This may have improved mood on subsequent occasions. Alternatively, a tryptophan- induced mood improvement may have helped FH+ individuals stand up for themselves at home, by increasing quarrelsomeness and decreasing agreeableness. Yet, whereas previous studies used a time-contingent EMA approach (i.e. assessment at fixed time points) (Gunthert et al., 2007; Vella et al., 2012), the EMA approach used in Study 2 (Hogenelst et al., 2015a) was event-contingent (e.g., depending on naturally occurring social interactions). The former approach is preferred for investigating the temporal dynamics of variables of interest because it involves fixed time intervals between measurements (Ebner-Priemer & Trull, 2012). Therefore, in the analyses of Study 2 I was unable to determine the direction of the relation between social behavior and mood, or vice versa.

7.4 Comparison of our findings with previous research

Facial emotion recognition and empathic accuracy

There are important differences between facial emotion recognition (FER) tasks and the empathic accuracy (EA) task that may explain the discrepancy between findings of previous studies (Harmer et al., 2003; Williams et al., 2007; aan het Rot et al., 2010; Beacher et al., 2011; Passamonti et al., 2012) and the results of Study 1. In Chapter 4 I already discussed that compared to FER tasks, in the EA task individuals can use multiple sources of information (e.g., visual, auditory, semantic) to make an inference of another persons' emotional state and thereby possibly overcome the negative effects of ATD on visual emotion perception. Perhaps measures which assess one source specifically versus measures which may be affected by a multitude of factors, are more sensitive to ATD. Another difference between FER tasks and the EA task pertains to the behavioral response that is required. In the FER task, individuals are asked to choose as quickly as possible among a fixed number of options to indicate the emotion (e.g., happy, angry, fearful) expressed in a briefly presented face (e.g., Harmer et al., 2003). In contrast, the EA task involves continuous affect ratings over time for each video clip (Zaki et al., 2008; aan het Rot & Hogenelst, 2014). ATD may impair the processing of specific information when time is limited and involves a series of rapid button presses. In contrast,

in the EA task responses are more continuous (e.g., from very negative to very positive) and there is no time pressure to respond. This may help explain why ATD did not impair performance on the EA task (Study 1) while it previously did impair performance in FER tasks (e.g., aan het Rot et al., 2010; Harmer et al., 2003).

Being quarrelsome may sometimes be beneficial

At first glance, an increase in quarrelsomeness and a decrease in agreeableness when taking tryptophan (Study 2) may seem disadvantageous. However, as stated in Chapter 6, the level to which tryptophan increased quarrelsome behavior in the FH+ individuals who were studied was not high. Further, the baseline level of agreeable behavior for FH+ individuals (i.e. during the placebo phase for participants who received tryptophan second) appeared similar to the baseline level of agreeable behavior that was found in a previous study for healthy participants (Moskowitz et al., 2001). These observations should be taken into consideration given that previous research has found higher levels of irritability in FH+ individuals compared to FH- individuals (Lauer et al., 1997).

Yet, I argued in Chapter 6 that the increase in quarrelsomeness and decrease in agreeableness seen during tryptophan administration may have helped FH+ individuals to stand up for themselves at home. Although this remains speculative, it may have increased control over their social environment. This would be in line with animal research suggesting that serotonin activity is positively related to behavior aimed at territorial control (de Boer et al., 2009). Moreover, in one study in healthy individuals, higher perceived control over situations predicted better subsequent mood (Ledrich & Gana, 2013). Nevertheless, it should be noted that there was no increase in dominance (as seen in Moskowitz et al., 2001). This might suggest that in Study 2, individuals may have used covert rather than overt behavioral means to change power or control.

Individual and cultural differences in the expression of dominance

In two previous studies involving healthy volunteers living in Canada, two weeks of tryptophan administration either increased dominant behavior (Moskowitz et al., 2001) or decreased dominant behavior in the male participants (aan het Rot et al., 2006). The increase in dominance observed in the first Canadian study was in line with Tse and Bond (2002b) and also predicted by studies conducted in nonhuman primates (e.g., Raleigh, McGuire, Brammer, Pollack, & Yuwiler, 1991). The decrease in dominance observed in the second Canadian study was unexpected, but it was argued that for the high-trait quar-

relsome men studied, the decrease in dominance may have increased liking from others and thus contributed to their enhanced social affiliation (cf. aan het Rot et al., 2006).

In Chapter 6, I showed that tryptophan did not significantly influence levels of dominant behavior in Dutch individuals at familial risk for depression (Hogenelst et al., 2015a). The results from previous studies (e.g., Moskowitz et al., 2001; aan het Rot et al., 2006) suggest that the effects of tryptophan on dominance in everyday social interactions may depend on who is taking tryptophan. It is possible that in Study 2 (Chapter 6), tryptophan increased dominance in some individuals, but decreased it in others leading to a net effect of no change in dominance.

In addition, there may be cultural differences in the expression of dominant behavior (Hofstede, 2001). Previous research has indicated that for people in North America, including Canada, it is considered more acceptable to be overtly assertive and focused on power, whereas Dutch individuals are generally less power oriented (Hofstede, 2001; aan het Rot et al., 2013). While speculative, it could be that a lower focus on power may have precluded any effects of tryptophan on dominance in Study 2 (Chapter 6).

Significant null findings

The results of Study 1 and Study 2 by and large did not support our hypotheses. One potential explanation is that the unexpected findings were due to methodological limitations such as a poor research design, limited power, and flawed measures. However, both studies employed double-blind placebo-controlled crossover designs which have great methodological strengths (6 & Bellamy, 2012; Crockett & Fehr, 2014). Further, as discussed in Chapters 4 and 6, the unexpected findings cannot easily be attributed to a lack of power. Furthermore, as mentioned in Chapters 4-6, the measures to assess social behavior in Studies 1 and 2 had previously been validated (e.g., Chartrand & van Baaren, 2009; Moskowitz, 1994; Zaki et al., 2008) and had mostly been shown to be sensitive to pharmacological manipulation (e.g., aan het Rot et al., 2006; Bartz et al., 2010; Stassen et al., 1998).

Based on the discussion of the methodology and critical interpretation of the data presented in this Thesis, I believe that a more convincing explanation for the findings of Studies 1 and 2 is that (a) in never-depressed individuals reduced levels of serotonin may have no effect on social behavior when assessed more naturalistically than in previous studies, and (b) tryptophan-induced increases in quarrelsomeness and decreases in agreeableness might be beneficial for FH+ individuals, at least for a period of two weeks. Overall, I believe the unexpected findings of Studies 1 and 2 may provide important information to meaningfully advance science and clinical practice.

7.5 Not rigor *versus* vigor, but rigor *and* vigor

A quandary faced by many scientists relates to having to choose between rigorous designs (i.e., experimental control) and vigorous designs (i.e., optimizing ecological validity) (Adler et al., 2012). Rigorous research designs emphasize maximizing precision of measures and controlling extraneous variables (Kelman, 1968). However, by creating experimental conditions that are "sterile" and controlled, it is possible that experimenters are studying a parameter that contributes little to real life social functioning (Zaki & Ochsner, 2012). I illustrated this in Chapter 3 (Hogenelst et al., 2015b) with a study in which the ability to recognize facial expressions in a laboratory task was unrelated to real-life social functioning (Janssens et al., 2012). A failure to relate real-life outcome to facial expression recognition does not necessarily imply that facial expression recognition measures are faulty; it could imply that it taps into an aspect of social functioning which cannot be assessed with a self-rated scale. Nevertheless, this example illustrates that more research is necessary to determine how data obtained using laboratory measures of social behavior predicts behavior during real-life social interaction.

Vigorous designs may involve both qualitative and quantitative approaches to fully capture both predictor and outcome measures and their actual operation in real-world settings (Kelman, 1968). Combining the assessment of facial emotion recognition with a more ecologically valid assessment of social behavior is one example of overcoming the quandary between rigor and vigor when studying the neurobiology of social behavior. For example, assessment of FER and EA may be done in the same participants to assess the extent to which the recognition of static facial emotion expressions relates to EA based on video stimuli. We did not include the assessment of FER in Study 1 due to time limitations of the ATD protocol. Moreover, adding an FER assessment to a test battery including assessment of speech, behavioral mimicry, mood questionnaires, and EA might have overburdened participants. Still, combining FER and EA in future research would be helpful in bridging the gap between rigor and vigor. Another example would be to combine the assessment of social behavior in the laboratory with the assessment of social behavior using EMA in the same study (see also Trull & Ebner-Priemer, 2013). In the current project this was not a feasible option because participating in Study 1 and Study 2 was too time-consuming for some FH+ individuals. Further, Study 1 and Study 2 involved different manipulations of serotonin. Nevertheless, it has been shown in an earlier study that administration of nutritionally-sourced tryptophan, relative to placebo, enhanced the perception of fearful and happy facial expressions after a single dose (Attenburrow et al., 2003). Prior to Study 2 we could have assessed the effect of tryptophan on FER, which would have allowed us to determine the extent to which the effect of an increase in serotonin on FER may translate to everyday social interactions.